

WHAT IS CLAIMED IS:

1. A method of determining the effect of a substance on characteristics of neurodegenerative disease in brain cells, said method comprising:

- (A) exposing brain cells to a condition that disrupts lysosomal activity or that increases cathepsin D in said cells,
- (B) maintaining said cells for a time sufficient to induce one or more characteristics of a neurodegenerative disease in said cells,
- (C) adding said substance before, during and/or after said exposing or said maintaining; and
- (D) determining whether the presence of said substance has an effect on said one or more said characteristics,

wherein said characteristics are selected from the group consisting of:

- (1) the formation of neurofibrillary tangles,
- (2) an increase in the phosphorylation of tau,
- (3) an increase in tau proteolytic fragments,
- (4) an increased production and/or release of brain-produced cytokines TGF-beta, IL-1b, TNF, or LPS,
- (5) an increased microglia reaction or microglial activation,
- (6) increased indications of brain inflammatory reactions,
- (7) increased conversion of p35 to p25,
- (8) increased activity of cyclin dependent protein kinase 5 (cdk5), and
- (9) increased levels of mitogen activated protein kinase (MAPK).

2. The method of claim 1, wherein said characteristic is an increase in the density of neurofibrillary tangles in said brain cells.

3. The method of claim 1, wherein said characteristic is an increase in the amount of phosphorylated tau in said brain cells.

4. The method of claim 1, wherein said characteristic is an increase in the amount of tau proteolytic fragments in said brain cells.

5. The method of claim 1, wherein said condition comprises contact said cells with an inhibitor of a lysosomal enzyme.

6. The method of claim 5, wherein said inhibitor is selected from the group consisting of a compound which is selected from the group consisting of chloroquine, N-CBZ-L-phenylalanyl-L-alanine-diazomethylketone, N-CBZ-L-phenylalanyl-L-phenylalanine-diazomethylketone, and beta-amyloid.

7. The method of claim 6, wherein said inhibitor is ZPAD.

8. The method of claim 1, wherein said brain cells are in the form of a brain slice.

9. The method of claim 7, wherein said brain slice is a hippocampal slice, an entorhinal cortex slice, an entorhinohippocampal slice, a neocortex slice, a hypothalamic slice, or a cortex slice.

10. The method of claim 1, wherein said brain cells are *in vivo*.

11. The method of any one of claims 1-10, wherein said brain cells are apolipoprotein E-deficient brain cells.

12. The method of any one of claims 1-10, wherein said brain cells are apolipoprotein E4-containing brain cells.

13. A method of determining the effect of a substance on characteristics of neurodegenerative disease in brain cells, said method comprising:

- (A) exposing brain cells to a condition that decreases an effective concentration of cholesterol in said cells,
- (B) maintaining said cells for a time sufficient to induce one or more characteristics of a neurodegenerative disease in said cells,
- (C) adding said substance before, during and/or after said exposing or said maintaining; and
- (D) determining whether the presence of said substance has an effect on said one or more characteristics,

wherein said characteristics are selected from the group consisting of:

- (1) the formation of neurofibrillary tangles,
- (2) an increase in the phosphorylation of tau,
- (3) an increase in tau proteolytic fragments,
- (4) an increased production and/or release of brain-produced cytokines TGF-beta, IL-1b or LPS,
- (5) an increased microglia reaction or microglial activation,
- (6) increased indications of brain inflammatory reactions,
- (7) decrease in the levels of p35,
- (8) decreased activity of cyclin dependent protein kinase 5 (cdk5), and
- (9) increased levels of mitogen activated protein kinase (MAPK).

14. The method of claim 13, wherein said characteristics comprise an increase in the density of neurofibrillary tangles in said brain cells.

15. The method of claim 13, wherein said characteristics comprise an increase in the amount of phosphorylated tau in said brain cells.

16. The method of claim 13, wherein said characteristics comprise an increase in the amount of tau proteolytic fragments in said brain cells.

17. The method of claim 13, wherein said condition comprises contacting said brain cells with an inhibitor of cholesterol synthesis.

18. The method of claim 13, wherein said condition comprises contacting said brain cells with a member of the family of compounds known as statins.

19. The method of claim 17, wherein said inhibitor is selected from the group consisting of mevastatin, simvastatin, atorvastatin, pravastatin, fluvastatin, lovastatin, cerivastatin, and mimetics thereof.

20. The method of claim 19, wherein said inhibitor is mevastatin.

21. The method of claim 13, wherein said brain cells are in the form of a brain slice.

22. The method of claim 21, wherein said brain slice is a hippocampal slice, an entorhinal cortex slice, an entorhinohippocampal slice, a neocortex slice, a hypothalamic slice, or a cortex slice.

23. The method of claim 13, wherein said brain cells are *in vivo*.

24. The method of claim 13, wherein said brain cells are apolipoprotein E-deficient brain cells.

25. The method of claim 13, wherein said brain cells are apolipoprotein E4-containing brain cells.

26. The method of claim 13, wherein said cells are also contacted with a cathepsin D-increasing compound.

27. A method of determining the effect of a substance on the inhibition of characteristics of neurodegenerative disease in brain cells, said method comprising:

- (A) exposing brain cells to a condition that disrupts lysosomal activity or that increases cathepsin D in said cells,
- (B) maintaining said cells for a time sufficient to induce one or more characteristics of a neurodegenerative disease in said cells,
- (C) adding a cysteine protease inhibitor before, during and/or after said exposing or said maintaining;
- (D) adding said substance before, during and/or after said exposing or said maintaining; and
- (E) determining whether the presence of said inhibitor has an effect on the inhibition of the development of said one or more characteristics, wherein said characteristics are selected from the group consisting of:
 - (1) the formation of neurofibrillary tangles,
 - (2) an increase in the phosphorylation of tau,
 - (3) an increase in tau proteolytic fragments,
 - (4) an increased production and/or release of brain-produced cytokines TGF-beta, IL-1b, TNF, or LPS,
 - (5) an increased microglia reaction or microglial activation,
 - (6) increased indications of brain inflammatory reactions,
 - (7) increased conversion of p35 to p25,

- (8) increased activity of cyclin dependent protein kinase 5 (cdk5), and
- (9) increased levels of mitogen activated protein kinase (MAPK).

28. The method of claim 27, wherein said characteristics comprise an increase in the density of neurofibrillary tangles in said brain cells.

29. The method of claim 27, wherein said characteristics comprise an increase in the amount of hyperphosphorylated tau in said brain cells.

30. The method of claim 27, wherein said characteristics comprise an increase in the amount of tau proteolytic fragments in said brain cells.

31. The method of claim 27, wherein said condition comprises an inhibitor of a lysosomal enzyme.

32. The method of claim 31, wherein said inhibitor is selected from the group consisting of a compound which is selected from the group consisting of chloroquine, N-CBZ-L-phenylalanyl-L-alanine-diazomethylketone, N-CBZ-L-phenylalanyl-L-phenylalanine-diazomethylketone, and beta-amyloid.

33. The method of claim 32, wherein said inhibitor is ZPAD.

34. The method of claim 27, wherein said brain cell is in the form of a brain slice.

35. The method of claim 34, wherein said brain slice is a hippocampal slice, an entorhinal cortex slice, an entorhinohippocampal slice, a neocortex slice, a hypothalamic slice, or a cortex slice.

36. The method of claim 27, wherein said brain cells are *in vivo*.

37. The method of claim 27, wherein said brain cells are apolipoprotein E-deficient brain cells.

38. The method of claim 27, wherein said brain cells are apolipoprotein E4-containing brain cells.

39. The method of claim 27, wherein said cysteine protease inhibitor is a calpain inhibitor.

40. A method of determining the effect of a substance on the inhibition of characteristics of neurodegenerative disease in brain cells, said method comprising:

- (A) exposing brain cells to a condition that decreases an effective concentration of cholesterol in said cells,
- (B) maintaining said cells for a time sufficient to induce one or more characteristics of a neurodegenerative disease in said cells,
- (C) adding a cysteine protease inhibitor before, during and/or after said exposing or said maintaining;
- (D) adding said substance before, during and/or after said exposing or said maintaining; and
- (E) determining whether the presence of said inhibitor has an effect on the inhibition of the development of said one or more characteristics,

wherein said characteristics are selected from the group consisting of:

- (1) the formation of neurofibrillary tangles,
- (2) an increase in the phosphorylation of tau,
- (3) an increase in tau proteolytic fragments,
- (4) an increased production and/or release of brain-produced cytokines TGF-beta, IL-1b, TNF, or LPS,

- (5) an increased microglia reaction or microglial activation,
- (6) increased indications of brain inflammatory reactions,
- (7) decrease in the levels of p35,
- (8) decreased activity of cyclin dependent protein kinase 5 (cdk5), and
- (9) increased levels of mitogen activated protein kinase (MAPK).

41. The method of claim 40, wherein said characteristics comprise an increase in the density of neurofibrillary tangles in said brain cells.

42. The method of claim 40, wherein said characteristics comprise an increase in the amount of phosphorylated tau in said brain cells.

43. The method of claim 40, wherein said characteristics comprise an increase in the amount of tau proteolytic fragments in said brain cells.

44. The method of claim 40, wherein said condition comprises contacting said brain cells with an inhibitor of cholesterol synthesis.

45. The method of claim 40, wherein said condition comprises contacting said brain cells with a member of the family of compounds known as statins.

46. The method of claim 44, wherein said inhibitor is selected from the group consisting of mevastatin, simvastatin, atorvastatin, pravastatin, fluvastatin, lovastatin, cerivastatin, and mimetics thereof.

47. The method of claim 46, wherein said inhibitor is mevastatin.

48. The method of claim 40, wherein said brain cells are in the form of a brain slice.

49. The method of claim 48, wherein said brain slice is a hippocampal slice, an entorhinal cortex slice, an entorhinohippocampal slice, a neocortex slice, a hypothalamic slice, or a cortex slice.

50. The method of claim 40, wherein said brain cells are *in vivo*.

51. The method of claim 40, wherein said brain cells are apolipoprotein E-deficient brain cells.

52. The method of claim 40, wherein said brain cells are apolipoprotein E4-containing brain cells.

53. The method of claim 40, wherein said cells are also contacted with a compound that increases cathepsin D.

54. A method for inhibiting tau proteolysis in brain cells, said method comprising contacting said cells with an effective concentrations of a cysteine protease inhibitor.

55. The method of claim 54, wherein said inhibitor is a calpain inhibitor.

56. The method of claim 55, wherein said calpain inhibitor inhibits calpain I.

57. The method of claim 55, wherein said calpain inhibitor inhibits calpain II.

58. The method of claim 54, wherein said inhibitor inhibits the production of the 15-35 kDa tau proteolytic fragments.

59. The method of claim 54, wherein said inhibitor inhibits the production of the 33 kDa tau proteolytic fragment.

60. A method of determining the effect of a substance on the inhibition of characteristics of neurodegenerative disease in brain cells, said method comprising:

- (A) exposing brain cells to a condition that disrupts lysosomal activity or that increases cathepsin D to a concentration effective to induce one or more characteristics of a neurodegenerative disease in said cells,
- (B) maintaining said cells for a time sufficient to induce said one or more characteristics of a neurodegenerative disease in said cells,
- (C) adding a mitogen activated kinase inhibitor before, during and/or after said exposing or said maintaining;
- (D) adding said substance before, during and/or after said exposing or said maintaining; and
- (E) determining whether said substance has an effect on the inhibition of said one or more characteristics,

wherein said characteristics are selected from the group consisting of:

- (1) the formation of neurofibrillary tangles,
- (2) an increase in the phosphorylation of tau,
- (3) an increase in tau proteolytic fragments,
- (4) an increased production and/or release of brain-produced cytokines TGF-beta, IL-1b, TNF, or LPS,
- (5) an increased microglia reaction or microglial activation,
- (6) increased indications of brain inflammatory reactions,

- (7) increased conversion of p35 to p25,
- (8) increased activity of cyclin dependent protein kinase 5 (cdk5), and
- (9) increased levels of mitogen activated protein kinase (MAPK).

61. The method of claim 60, wherein said characteristics comprise an increase in the density of neurofibrillary tangles in said brain cells.

62. The method of claim 60, wherein said characteristics comprise an increase in the amount of hyperphosphorylated tau in said brain cells.

63. The method of claim 60, wherein said characteristics comprise an increase in the amount of tau proteolytic fragments in said brain cells.

64. The method of claim 60, wherein said inhibitor is a MAP kinase inhibitor.

65. The method of claim 60, wherein said inhibitor is selected from the group consisting of PD98059, SB203580 and U0126.

66. The method of claim 66, wherein said inhibitor is PD98059.

67. The method of claim 60, wherein said brain cell is in the form of a brain slice.

68. The method of claim 67, wherein said brain slice is a hippocampal slice, an entorhinal cortex slice, an entorhinohippocampal slice, a neocortex slice, a hypothalamic slice, or a cortex slice.

69. The method of claim 60, wherein said brain cells are *in vivo*.

70. The method of claim 60, wherein said brain cells are apolipoprotein E-deficient brain cells.

71. The method of claim 60, wherein said brain cells are apolipoprotein E4-containing brain cells.

72. A method of determining the effect of a substance on the inhibition of characteristics of neurodegenerative disease in brain cells, said method comprising:

- (A) exposing brain cells to a condition that decreases an effective concentration of cholesterol in said cells,
- (B) maintaining said cells for a time sufficient to induce one or more characteristics of a neurodegenerative disease in said cells,
- (C) adding a mitogen activated kinase inhibitor before, during and/or after said exposing or said maintaining;
- (D) adding said substance before, during and/or after said exposing or said maintaining; and
- (E) determining whether the presence of said inhibitor has an effect on the inhibition of the development of said one or more characteristics,

wherein said characteristics are selected from the group consisting of:

- (1) the formation of neurofibrillary tangles,
- (2) an increase in the phosphorylation of tau,
- (3) an increase in tau proteolytic fragments,
- (4) an increased production and/or release of brain-produced cytokines TGF-beta, IL-1b, TNF, or LPS,
- (5) an increased microglia reaction or microglial activation,
- (6) increased indications of brain inflammatory reactions,
- (7) decrease in the levels of p35,

- (8) decreased activity of cyclin dependent protein kinase 5 (cdk5), and
- (9) increased levels of mitogen activated protein kinase (MAPK).

73. The method of claim 72, wherein said characteristics comprise an increase in the density of neurofibrillary tangles in said brain cells.

74. The method of claim 72, wherein said characteristics comprise an increase in the amount of phosphorylated tau in said brain cells.

75. The method of claim 72, wherein said characteristics comprise an increase in the amount of tau proteolytic fragments in said brain cells.

76. The method of claim 72, wherein said condition comprises contacting said brain cells with an inhibitor of cholesterol synthesis.

77. The method of claim 72, wherein said condition comprises contacting said brain cells with a member of the family of compounds known as statins.

78. The method of claim 76, wherein said inhibitor of cholesterol synthesis is selected from the group consisting of mevastatin, simvastatin, atorvastatin, pravastatin, fluvastatin, lovastatin, cerivastatin, and mimetics thereof.

79. The method of claim 78, wherein said inhibitor is mevastatin.

80. The method of claim 72, wherein said brain cells are in the form of a brain slice.

81. The method of claim 80, wherein said brain slice is a hippocampal slice, an entorhinal cortex slice, an entorhinohippocampal slice, a neocortex slice, a hypothalamic slice, or a cortex slice.

82. The method of claim 72, wherein said brain cells are *in vivo*.

83. The method of claim 72, wherein said brain cells are apolipoprotein E-deficient brain cells.

84. The method of claim 72, wherein said brain cells are apolipoprotein E4-containing brain cells.

85. The method of claim 72, wherein said cells are also contacted with a compound that increases cathepsin D.

86. The method of claim 72, wherein said mitogen activated kinase inhibitor is a MAP kinase inhibitor.

87. The method of claim 72, wherein said mitogen activated kinase inhibitor is selected from the group consisting of PD98059, SB203580 and U0126.

88. The method of claim 86, wherein said mitogen activated kinase inhibitor is PD 98059.

89. A method of intervening in the process of neurodegeneration due to the proteolysis of tau occurring in the neural tissue of a mammal, said method comprising administering to said mammal a pharmaceutically acceptable form of a cysteine protease inhibitor or a pharmaceutically acceptable salt thereof.

90. The method of claim 88, wherein said inhibitor is a calpain inhibitor.

91. The method of claim 89, wherein said inhibitor inhibits calpain I.
92. The method of claim 89, wherein said inhibitor inhibits calpain II.
93. The method of claim 88, wherein said inhibitor inhibits the production of 15-35 kDa tau proteolytic fragments.
94. The method of claim 91, wherein said proteolytic fragment is 33 kDa.

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25